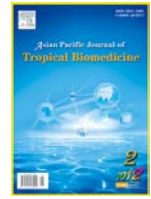




Contents lists available at ScienceDirect

## Asian Pacific Journal of Tropical Biomedicine

journal homepage: [www.elsevier.com/locate/apjtb](http://www.elsevier.com/locate/apjtb)

Document heading

## Antioxidant activity of *Palmarosa essential oil (Cymbopogon martini)* grown in north Indian plains

Kapil Lawrence<sup>1</sup>, Reena Lawrence<sup>2\*</sup>, Dharmendra Parihar<sup>3</sup>, Rashmi Srivastava<sup>4</sup>, Abhishek Charan<sup>1</sup><sup>1</sup>Department of Biochemistry & Bioprocess Technology, Sam Higginbottom Institute of Agriculture, Technology and Sciences, (Formerly AAI–DU), India<sup>2</sup>Department of Chemistry, Sam Higginbottom Institute of Agriculture, Technology and Sciences (Formerly AAI–DU), Allahabad, India<sup>3</sup>Department of Biotechnology, Guru Ghasidas Vishwavidyalaya (Central University), Bilaspur, Chattisgarh, India<sup>4</sup>Biotech Research Institute, Lucknow, U.P, India

## ARTICLE INFO

## Article history:

Received 14 June 2012

Received in revised form 5 July 2012

Accepted 7 August 2012

Available online 28 August 2012

## Keywords:

Palmarosa oil

BHT

Gallic acid

Antioxidant activity

## ABSTRACT

**Objective:** The present study deals with the *in vitro* study of antioxidant activity of essential oil from *Palmarosa (Cymbopogon martini)* of family gramineae. **Methods:** The *in vitro* study of antioxidant activity of *Palmarosa* essential oil has been done by using DPPH assay, Nitrogen oxide assay, reducing power assay,  $\beta$ -carotene bleaching assay and FRAP method. **Results:** IC<sub>50</sub> values observed for DPPH and NO assay were 0.125 mg/mL and 12.5  $\mu$ g/mL, respectively. In beta carotene bleaching method, the oil showed 93.15% bleaching for the first hour and it increased to 51.1% in second hour. There was a constant increase in the reducing activities with the increase in concentrations in both reducing activity and FRAP methods. In all the methods BHT and Gallic acid were kept as standards. **Conclusions:** The results clearly indicate that *Palmarosa* essential oil is effective in scavenging free radical and has the potential to be powerful antioxidant.

### 1. Introduction

An essential oil is a liquid that is generally distilled (more frequently by steam or water) from the leaves, stems, flowers, bark or roots or other elements of the plant[1]. These are also known as volatile or ethereal oils. Medicinal applications of essential oils range from skin treatment to remedies for cancer.

*Palmarosa* oil is extracted from *Cymbopogon martini* of family gramineae and is also known as Turkish geranium as well as Indian rosha and motia. It is a sweet floral with a hint of rose smell and is pale yellow in color with nearly watery viscosity. The yield of oil is generally 1.0%–1.5%. Essential oil is extracted from inflorescence (2%), leaf lamina (1.4%), whole herb (0.75%) and leaf sheath (0.33%). The major constituents of oil of whole herb are linalool,  $\beta$ -caryophyllene and E, Z-farnesol[2].

*Palmarosa* oil had a long history of use in India

and Turkey. In aromatherapy the oil is reputed to be regenerative and extremely effective against pathogenic intestinal flora to turn them back to normal cell or Intestinal mucous membrane[3,4]. Its most important use is in the treatment of skin infections like acne and also to stimulate cell regeneration while regulating the production of sebum. It may be beneficial for cardiovascular system, circulation, digestion, infection, nervous system and rashes.

Apart from all the above use the essential oil has been shown to be effective insect repellent when applied to stored grains and beans and as antihelmentic against nematodes[5,6]. There are other activities also reported for oil like antifungal[7] and antimicrobial[8–10].

Antioxidants in food play an important role as a health protecting factor. Scientific evidence suggest that antioxidants reduce the risk for chronic diseases. The main characteristic of an antioxidant is its ability to trap free radicals. Highly reactive free radicals and oxygen species are present in biological systems from a wide variety of sources. These free radicals may oxidize nucleic acid, protein lipid or DNA and can initiate degenerative diseases. Antioxidant (compounds) like phenolic acids, polyphenols and flavonoids scavenge free radicals such as peroxides, hydroperoxides or lipid peroxy and thus inhibit the oxidative mechanism that lead to degenerative diseases.

\*Corresponding author: Reena Lawrence, Department of Chemistry, Sam Higginbottom Institute of Agriculture, Technology and Sciences (Formerly AAI–DU), Allahabad, India.  
Tel: +91-9792088269  
Fax: +91-532-2684012  
E-mail: reena\_lawrence74@yahoo.co.in

Although there are some synthetic antioxidants also like BHT and BHA but all these are associated with some side effects. A majority of plant species has been investigated time to time for their use as antioxidant and antimicrobial agents. Since the biological activities of essential oils usually varies depending on the place where they are grown so our study aims at the study of antioxidant activities of essential oil obtained from *Palmarosa* plant grown in North Indian plains<sup>[11,12]</sup>. For this purpose antioxidant activity has been checked by the following methods: DPPH assay, Reducing activity assay,  $\beta$ -carotene bleaching assay, FRAP method and Nitrogen oxide method.

## 2. Material and methods

The essential oil has been obtained from Central Institute of Medicinal and Aromatic Plants in April 2011. The specimen voucher has been deposited at our University herbarium.

All the chemicals used in the work were purchased from HI-MEDIA Pvt. Ltd, Bombay. The chemicals used were of analytical grade.

### 2.1. Determination of antioxidant activity

The antioxidant activity was evaluated by five methods: DPPH assay, reducing antioxidant activity, NO assay, Beta-carotene method and FRAP assay.

### 2.2. Free radical scavenging activity (DPPH method)

The hydrogen atom or electron donating ability of essential oil and standards—Gallic Acid and BHT was determined from bleaching of purple colored methanol solution of DPPH. This spectrophotometric assay uses the stable radical DPPH as a reagent. The diluted working solution of essential oil were prepared in methanol (2.0000, 1.0000, 0.5000, 0.2500, 0.1250 and 0.0625 mg/mL). DPPH was prepared at a concentration of 0.002%. Different concentrations of essential oils was taken in each test tube and volumes was made upto 2 mL. Then 2 mL of DPPH solution was added in each test tube and these solutions were kept in dark for 30 min. The same procedure was followed for BHT and Gallic Acid. All the samples were tested in triplicate. Later optical density was recorded at 517 nm using UV-Visible spectrophotometer. Methanol with DPPH was used as control. The method was same as used by Kahalaf *et al*<sup>[13]</sup> with slight modification. The formula used for calculation is:

$$\% \text{ Inhibition of DPPH activity} = (A-B/A) \times 100$$

Where A—Optical density of control  
B—Optical density of sample.

### 2.3. Reducing power

The reducing antioxidant activity of the essential oil has been analyzed by the method given by Huda Fajan *et al*<sup>[14]</sup> with slight alterations. In this method different concentrations of essential oil (2.000, 1.000, 0.500, 2.500 and 0.125  $\mu\text{g/mL}$ ) were taken in different tubes and volume of all the working solutions is made upto 1 mL by adding distilled water, in these added 2.5 mL Phosphate buffer

(0.2 M, pH 6.6) and 2.5 mL of potassium ferricyanide (1%). The mixture was incubated for 20 min. at 50 °C. Then 2.5 mL TCA (Trichloroacetic acid, 10%) was added to each mixture and these were centrifuged for 10 min. at 3000 r/min. Then 2.5 mL of the upper layer was mixed with distilled water (2.5 mL) and 0.5 mL  $\text{FeCl}_3$  (0.1%) solution. Then absorbance was measured at 700 nm against a blank using UV-Visible spectrophotometer. The same procedure was repeated with gallic acid and BHT used as standard and sample without the oil was used as control. Increased absorbance of reaction mixture indicated increase in reducing power.

### 2.4. Nitric oxide scavenging activity method

Nitric oxide was generated from sodium nitroprusside (SNP) and was measured by Griess reagent. SNP in aqueous solution at physiological pH spontaneously generates NO, which interacts with oxygen to produce nitrite ion that can be estimated by use of Griess Reagent, Sodium nitroprusside (5 mM) in phosphate buffer saline (PBS) was mixed with different concentrations (100.00, 50.00, 25.00, 12.50 and 0.62  $\mu\text{g/mL}$ ) respectively and volume was made upto 3.0 mL. The solution was kept at 25 °C for 180 min. Then the sample from the above were reacted with Griess reagent (a solution of 1% sulphanilic acid in 2% phosphoric acid and 0.1% naphthylamine in distilled water). The absorbance of the chromophore produced by diazotization of nitrite ion with sulphanilic acid and subsequent coupling with naphthylamine was read a 546 nm. BHT and gallic acid were used as standards. The method used has been taken from Ghosh *et al*<sup>[15]</sup>. The formula used for calculation is:

$$\text{Nitric oxide scavenged (\%)} = (A-B/A) \times 100$$

Where A—Optical density of control  
B—Optical density of sample of essential oil.

### 2.5. $\beta$ -carotene bleaching method

The method followed is same as used by Geckil *et al*<sup>[16]</sup> with some moderations. 0.02 mg of crystalline  $\beta$ -carotene was dissolved in 10 mL of chloroform and then added in this 20 mg linoleic acid and 200 mg of Tween-80 reagent (Merck). Chloroform was removed in rotary evaporator under vacuum at 40 °C for 5 min and then 50 mL of dist. Water was added with vigorous stirring to form an emulsion. Five mL of this emulsion was taken and added in 0.1 mL of essential oil extract (1  $\mu\text{g/mL}$ ). BHT was used as standard. The test tube containing sample and standard were kept in water bath in incubator at 50 °C and absorbance was recorded at an interval of 15 min till 2 h at 470 nm.

### 2.6. Ferric reducing antioxidant power (FRAP) assay

The method for determining the ferric reducing ability of the essential oil has been taken in modified form from the method used by Chaiyasut *et al*<sup>[17]</sup> and Jukic *et al*<sup>[18]</sup>. Briefly FRAP reagent has been prepared by mixing 0.1 M Acetate buffer (pH 3.6) with 10 mM 2, 4, 6-tripyridyl-s-triazine (TPTZ) and 20 mM ferric chloride in a ratio of 10:1:1 (v:v:v), Then 3 mL of the Frap reagent has been added to 0.1, 0.2, 0.4, 0.6, 0.8 and 1.0 mM of samples of essential oil (taken in triplicate), and left at room temperature for 5 min. until the reaction was complete. The reacted sample were read at 593 nm using standard ferrous sulphate (with same

concentration) as standard. An increase in the absorbance of the reaction mixture indicate an increase in the reducing power of the sample.

### 3. Results

In order to determine the effect of concentration on radical scavenging power by DPPH method, five different working solutions were used (2,000, 1,000, 0,500, 0,250 and 0,125 mg/mL). Results showed (Figure 1) that percentage inhibition is in increasing order with the increase in concentration. IC<sub>50</sub> for *Palmarosa* oil was found to be 0,125 mg/mL.

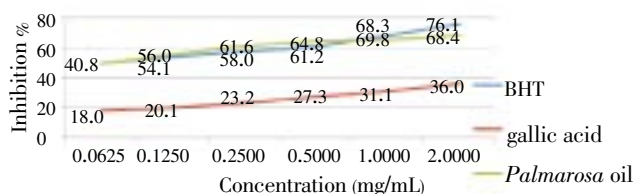


Figure 1. DPPH scavenging activity of BHT, gallic acid and essential oil of *Palmarosa*.

Reducing power characteristic of any compound serves as a significant indicator of its potential as antioxidant and is a supporting feature for its antioxidant activity. The concentrations used were 2,000, 1,000, 0,500, 2,500 and 0,125 µg/mL and absorbance was read at 700 nm. Reducing power was found to be significant (P<0.01). The results were found to be better as compared to the standards gallic acid. The activities were statistically (Figure 2) significant when compared with control.

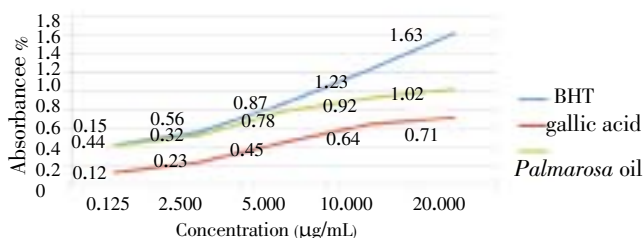


Figure 2. Reducing activity assay with BHT, gallic acid and *Palmarosa* oil.

Nitric oxide radical generated from Nitroprusside at physiological pH was found to be inhibited by the essential oil as shown in Figure 3. Gallic acid and BHT were used as reference. IC<sub>50</sub> value has been found to be 12,5 µg/mL for the sample.

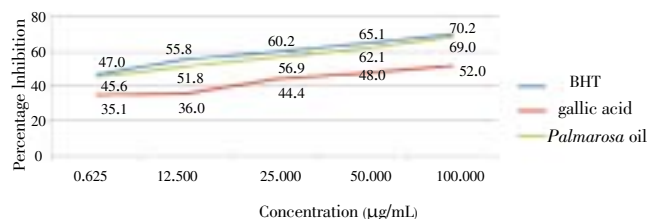


Figure 3. Nitric oxide scavenging assay with BHT, gallic acid and essential oil of *Palmarosa*.

The anti-bleaching activity of sample of β-carotene was studied by monitoring the color intensity of emulsion at 470 nm for every 15 min for 2 h. The concentration taken was 1 µg/mL for the sample as well as standard (BHT). The initial concentration was considered to be 100%. In the first 15 min the sample showed 93.1% bleaching as compared to 86.0% to that of standard. In 1 h of incubation, percentage decrease was found to be 71.8% and 55.1% for oil and standard respectively. During the second hour it came to 51.1% and 25.0% for the oil and standard, respectively (Figure 4).

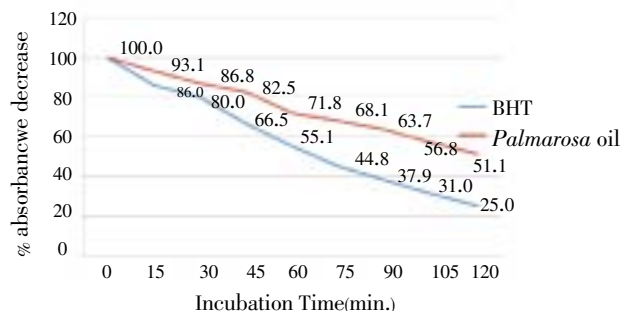


Figure 4. Relative changes in absorbance of beta carotene emulsions containing BHT and *Palmarosa* essential oil.

For FRAP assay concentration of the sample and standard FeSO<sub>4</sub> were taken to be 0,1, 0,2, 0,4, 0,6, 0,8 and 1,0 mM, the increase in absorbance with the concentration indicated increase in antioxidant activity (Figure 5). The FRAP assay measures the change in absorbance at 593 nm due to the formation of the blue colored Fe(II)-tripyridyltriazine compound from the colorless oxidized Fe(III) form by the action of electron donating antioxidants.

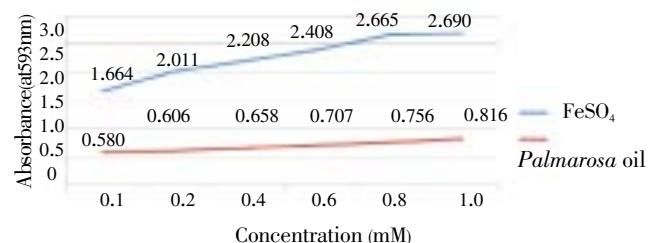


Figure 5. FRAP assay showing antioxidant activity of *Palmarosa* oil and FeSO<sub>4</sub>.

### 4. Discussion

Since the study has been done on *Palmarosa* essential oil and the grass is basically grown in Northern part of India and is indigenous so not much work has been done on the oil. One of the report has been given by Scherer R et al<sup>[12]</sup> who worked on the chemical composition, antioxidant and antimicrobial activity of the oil of clove (*Caryophyllum aromaticum* L.), *Citronella* (*Cymbopogon winterianus*) and *Palmarosa* (*Cymbopogon martini*). Antioxidant activity has been evaluated by DPPH (2,2-diphenyl-1-picrylhydrazyl) method. Clove oil showed the highest antioxidant activity, *Citronella* and *Palmarosa* oil presented low antioxidant activity.

Not much work has been done either on the essential oil or extracts from *Palmarosa* but antioxidant activity has been reported for different members of family Graminaea for example the antioxidant activity has been reported for *Vetiver* (*Vetiveria zizanioides*) oil by Kim HJ *et al*[19] by two methods namely DPPH free radical scavenging assay and Fe<sup>2+</sup> metal chelating assay and the results show a strong free radical scavenging activity when compared to standard BHT and  $\alpha$ -tocopherol. For example free radical scavenging activity for  $\alpha$ -tocopherol is 97% and 93% by metal chelating capacity and DPPH methods and same is 34% and 89% for *Vetiver* essential oil, respectively.

Another team work on *Vetiver* root oil has been done by Luqman *et al*[20], they have taken essential oil from *Vetiver* root of two species KSI and Gulabi. The antioxidant activity has been checked by FRAP, DPPH and reducing power assay. Out of the two KSI showed higher FRAP values, DPPH inhibition and reducing power.

Antioxidant activity has also been studied on another member of Graminaea *i.e* lemon grass (*Cymbopogon citratus*) which is also a native of north India. Study has been done on Citral which is the major constituent of lemongrass by Rabbani SI *et al*[21] who have tested antioxidant activity by superoxide scavenging method, the result indicated that citral significantly ( $P < 0.01$ ) inhibited the formation of micronuclei induced by Nickel and superoxide scavenging activity ( $EC_{50} = 19$  mcg/mL) was observed in citral treated groups.

Another scientist Salem AM *et al*[22] compared the antioxidant and antibacterial activity of garlic, thyme and lemongrass oils. Antioxidant activity has been tested by TBA method it was found that best results were obtained at 1.5% and activity was in the order lemongrass oil > thyme > garlic oil.

From the discussion, it can be seen that this is the first report on antioxidant activity of *Palmarosa* oil obtained in north Indian plains. *Palmarosa* oil showed strong antioxidant activity by inhibiting DPPH and NO radicals. Reducing power assay, FRAP and beta-carotene bleaching assay also showed similar results. The activities are comparable to standard antioxidants. So by such study it can be seen that *Palmarosa* oil can be taken as easy source of natural antioxidants

## References

- [1] Rao BRR, Rajput DK, Patel RP. Essential oil profiles of different parts of *Palmarosa* (*Cymbopogon martini*) (Roxb.) Wats var. motia Burk. *J Essential Oil Res* 2009; 11.
- [2] Randriamiharisoa RP, Caydou EM. Composition of *Palmarosa* (*Cymbopogon martini*) essential oil from Madagascar. *J Agri Food Chem* 1987; 35: 62–65.
- [3] Jirovetz L, Eller G, Buchbauer G, Schimidt E. Chemical composition, antimicrobial activities and odor description of some essential oils with characteristic floral rosy scent and of the principal aroma compounds. *Recent Res Dev Agron Horticulure* 2006; 2: 1–12.
- [4] Chen W, Viljoen AM. Geraniol—A review of a commercially important fragrance material. *South African J Botany* 2010; 76: 643–651.
- [5] Kumar R, Srivastava M, Dubey NK. Evaluation of *Cymbopogon martini* oil extract for control of post harvest insect deterioration in cereals and legumes. *J Food Protect* 2007; 70: 172–178.
- [6] Kumar AM, D'Souza P, Agarwal A, Bokkolla RM, Balasubramaniam M. Geraniol, the putative anthelmintic principle of *Cymbopogon martini*. *Phytotherapia Res* 2003; 17: 957–959.
- [7] Saikia D, Khanuja SPS, KaholAP, Gupta SC, Kumar S. Comparative antifungal activity of essential oils and constituents from three distinct genotype of *Cymbopogon* species. *Current Science* 2001; 80: 1264–1266.
- [8] Lodhia MK, Bhatt KR, Thaker VS. Antibacterial activity of essential oil from *Palmarosa*, evening primrose, lavender and *Tuberose*. *Ind J Pharm Sci* 2009; 71: 134–136.
- [9] Parashar A, Hili P, Robert GV, Evans CS. Antimicrobial action of *Palmarosa* oil (*Cymbopogon martini*) in *Sacchomyces cerevisiae*. *Phytochem* 2003; 63: 569–575.
- [10] Sonboli A, Mirjalili MH, Yousefzadi M. Antibacterial activity and composition of essential oil of *Cymbopogon olivieri* (Boss) bor from Iran. *Iran J Pharm Res* 2006; 1: 65–68.
- [11] Patnaik S, Subramanyam VR, Kole C. Antibacterial and antifungal activity of ten essential oil *in vitro*. *Microbios* 1996; 86: 237–246.
- [12] Scherer R, Wagner R, Duarte MCT, Godoy HT. Composition, antioxidant and antimicrobial activities of clove *Citronella* and *Palmarosa* essential oil. *Revist Brasileira de plantas Medicinaiis* 2009; 11: 442–449.
- [13] Khalaf NA, Shakya AK, Othman AA, El-Agbar Z, Farah H. Antioxidant activity of some common plants. *Turk J Biol* 2008; 32: 51–55.
- [14] Huda–Faujan N, Noriham A, Norrakiah AS, Babji AS. Antioxidant activity in plants ethanol extract containing phenolic compounds. *Afric J Biotechnol* 2009; 8: 484–489.
- [15] Ghosh R, Kadam P, Kadam V. *In vitro* antioxidant activity of “Kumbhajatu”—A polyphenol formulation. *Int J Pharm Res Dev* 2010; 1(11).
- [16] Geckil H, Ates B, Durmaz G, Erdogan S, Yilmaz I. Antioxidant, free radical scavenging and metal chelating characteristic of Propolis. *American J Biochem Biotechnol* 2005; 1: 27–31.
- [17] Chaiyasut C, Kumar T, Tipduangta P, Rungsevijitprapa W. Isofavone content and antioxidant activity of Thai fermented soyabean and its capsule formulation. *Afric J Biotechnol* 2010; 9: 4120–4126.
- [18] Juckic M, Milos M. Catalytic oxidation and antioxidant properties of Thyme essential oil (*Thymus vulgaris* L). *Croatica Chemica Acta* 2005; 78: 105–110.
- [19] Kim HJ, Chen F, wang Xi, Cheng HY, Zhengyn J. Evaluation of anioxidant activity of *Vetiveria zizanioides* L oil and identification of its antioxidant constituents. *J Agri Food Chem* 2005; 53: 7691–7695.
- [20] Luqman S, Kumar R, Kaushik S, Srivastava S, Darokar MP, Khanuja SPS. Antioxidant potential of roots of *Vetiveria zizanioides* L Nash. *Ind J Biochem Biophysics* 2009; 46: 122–125.
- [21] Rabbani SI, Devi K, Khanam S, Zahra N. Citral, a component of lemon grass oil inhibits the clastogenic effect of Nickel chloride in mouse micronucleus test system. *Pak J Pharm Sci* 2006; 19: 108–113.
- [22] Salem AM, Amin RA, Afifi GSA. Studies on antimicrobial and antioxidant activity efficiency of some essential oil in minced beef. *J Am Sci* 2010; 6: 691–700.